510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k112545

B. Purpose for Submission:
New Device

C. Measurand:
Anti-Serine Proteinase-3 Immunoglobulin G (IgG) Antibodies (PR3)
Anti-Myeloperoxidase Immunoglobulin G (IgG) Antibodies (MPO)
Anti-Glomerular Basement Membrane Immunoglobulin G (IgG) Antibodies (GBM)

D. Type of Test:
Semi-Quantitative, Chemiluminescent Immunoassays (CIA)

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA Flash® PR3 Reagents
QUANTA Flash® PR3 Calibrators
QUANTA Flash® PR3 Controls

QUANTA Flash® MPO Reagents
QUANTA Flash® MPO Calibrators
QUANTA Flash® MPO Controls

QUANTA Flash® GBM Reagents
QUANTA Flash® GBM Calibrators
QUANTA Flash® GBM Controls

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5660, Multiple Autoantibodies Immunological Test System
   21 CFR §862.1150, Calibrator
   21 CFR §862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:
   Class II – Assay and Calibrator
   Class I – Quality Control Material

3. Product code:
   MOB – Test System, Anti-Neutrophil Cytoplasmic Antibody (ANCA)
   MVJ – Devices, Measure, Antibodies to Glomerular Basement Membrane (GBM)

JIX – Calibrator, Multi-Analyte Mixture
JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:
   Immunology (82)
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

QUANTA Flash PR3
QUANTA Flash® PR3 Reagents is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG anti-proteinase 3 (PR-3) autoantibodies in human serum on the BIO-FLASH® instrument. QUANTA Flash® PR3 is an aid in the diagnosis of granulomatosis with polyangiitis (GPA) in conjunction with clinical findings and other laboratory tests.

The QUANTA Flash® PR3 Calibrators are intended for use with the QUANTA Flash® PR3 chemiluminescent immunoassay (CIA) on the BIO-FLASH® instrument. Each calibrator establishes a point of reference for the working curve that is used in the measurement of IgG anti-proteinase 3 (PR3) autoantibodies in human serum.

The QUANTA Flash® PR3 Controls are intended for quality control purposes of the QUANTA Flash® PR3 chemiluminescent immunoassay (CIA) kit run on a BIO-FLASH® instrument that is used in the measurement of IgG anti-proteinase 3 (PR3) autoantibodies in human serum.

QUANTA Flash MPO
QUANTA Flash® MPO Reagents is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG anti-myeloperoxidase (MPO) autoantibodies in human serum on the BIO-FLASH® instrument. QUANTA Flash® MPO is an aid in the diagnosis of microscopic polyangiitis (MPA) in conjunction with clinical findings and other laboratory tests.

The QUANTA Flash® MPO Calibrators are intended for use with the QUANTA Flash® MPO chemiluminescent immunoassay (CIA) on the BIO-FLASH® instrument. Each calibrator establishes a point of reference for the working curve that is used in the measurement of IgG anti-myeloperoxidase (MPO) autoantibodies in human serum.

The QUANTA Flash® MPO Controls are intended for quality control purposes of the QUANTA Flash® MPO chemiluminescent immunoassay (CIA) kit run on a BIO-FLASH® instrument that is used in the measurement of IgG anti-myeloperoxidase (MPO) autoantibodies in human serum.

QUANTA Flash GBM
QUANTA Flash® GBM Reagents is a chemiluminescent immunoassay (CIA) for the

semi-quantitative detection of IgG anti-glomerular basement membrane (GBM) autoantibodies in human serum on the BIO-FLASH® instrument. QUANTA Flash® GBM is an aid in the diagnosis of Goodpasture's Syndrome in conjunction with clinical findings and other laboratory tests.

The QUANTA Flash® GMP Calibrators are intended for use with the QUANTA Flash® GMP chemiluminescent immunoassay (CIA) on the BIO-FLASH® instrument. Each calibrator establishes a point of reference for the working curve that is used in the measurement of IgG anti-glomerular basement membrane (GBM) autoantibodies in human serum.

The QUANTA Flash® GBM Controls are intended for quality control purposes of the QUANTA Flash® GBM chemiluminescent immunoassay (CIA) kit run on a BIO-FLASH® instrument that is used in the measurement of IgG anti-glomerular basement membrane (GBM) autoantibodies in human serum.

2. Indication(s) for use:
   Same as Intended use

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   BIO-FLASH® chemiluminescent analyzer (k101644)

I. Device Description:

Materials Provided:

QUANTA Flash® Reagent cartridge contains one reagent cartridge with sufficient material for 50 tests. Each cartridge has a barcode that contains the assay name, the assay ID number, the lot number and expiration date, the four parameters of the lot specific master curve, and the reagent cartridge specific serial number. The QUANTA Flash® reagent cartridge contains 4 reagent tubes:

- PR3/MPO/GBM coated paramagnetic beads in a buffered solution containing protein stabilizers and preservative;
- Assay buffer – phosphate-buffered saline solution containing Tween 20, protein stabilizers and preservatives;
- Tracer IgG – Isoluminol labeled anti-human IgG antibodies in a buffered solution containing protein stabilizers and preservative;
- The fourth reagent tube is empty for QUANTA Flash® PR3 and GBM since the sample dilution is done with system rinse from the system; for QUANTA Flash® MPO the fourth reagent tube (colored pink) contains sample dilution buffer containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.

Materials required but not provided:

- BIO-FLASH® instrument with operating computer;
- BIO-FLASH® System Rinse contains four 5-liter bottles of phosphate buffered saline

with Tween-20 and sodium azide;
- BIO-FLASH® Triggers contains one bottle each of Trigger 1 (the catalyst) and 2 (the oxidant);
- BIO-FLASH® Cuvettes;
- QUANTA-FLASH® Calibrators;
- QUANTA-FLASH® Controls.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) numbers:

Predicative device	Predicate 510(k) number(s)
QUANTA Lite® PR3 ELISA	k981328
QUANTA Lite® MPO ELISA	k981330
QUANTA Lite® GBM ELISA	k984336

2. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use: QUANTA Flash® PR3/MPO Reagents	QUANTA Flash® PR3/MPO is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG anti-PR3/MPO autoantibodies in human serum on the BIO-FLASH® instrument. QUANTA Flash® PR3/MPO is an aid in the diagnosis of granulomatosis with polyangiitis (GPA)/ microscopic polyangiitis (MPA) in conjunction with clinical findings and other laboratory tests.	Same
QUANTA Flash® GBM Reagents	QUANTA Flash® GBM is a chemiluminescent immunoassay (CIA) for the semi-quantitative detection of IgG anti-glomerular basement membrane (GBM) autoantibodies in human	

Similarities		
Item	Device	Predicate
	serum on the BIO-FLASH® instrument. QUANTA Flash® GBM is an aid in the diagnosis of Goodpasture's Syndrome in conjunction with clinical findings and other laboratory tests.	
Assay Type	Semi-quantitative immunoassay	Same
Analyte Detected	Human IgG PR3/MPO/GBM autoantibodies	Same
Sample Matrix	Serum	Same
Antigen	Native purified antigen	Same
Cutoff between positive and negative	20 CU	Same

Differences		
Item	Device	Predicate
Assay Technology	Chemiluminescent Immunoassay (CIA) utilizing magnetic particles	Enzyme-linked Immunosorbent Assay (ELISA)
Conjugate	Isoluminol conjugated monoclonal anti-human IgG	Horse radish peroxidase conjugated goat anti-human IgG antibodies
Signal Detected	Luminescence (visible light)	Absorbance at 450nm
Calibration and unit calculation	Instrument specific working curve based off a 6 point lot specific master curve used for unit calculations; stored on the instrument.	Single point calibrator run each time the assay is run
Assay range: PR3 MPO GBM	 2.3 to 3,285.3 CU 3.2 to 739.8 CU 2.9 to 1,437.8 CU	N/A

K. Standard/Guidance Document Referenced (if applicable):

- CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.

- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.

L. Test Principle:

The QUANTA Flash® assays utilize a predefined lot specific Master Curve that is uploaded into the instrument through the reagent cartridge barcode. Based on the results of running two calibrators, an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the Relative Light Units (RLU) obtained for each patient. The calibrators are not included as part of the QUNTA Flash® assays.

The principles of the assay are similar to other solid phase indirect immunosorbent assays. The solid phase is paramagnetic beads and the detecting reagent is an isoluminol-conjugated anti-human IgG monoclonal antibody. Native human PR3, MPO or GBM are coated onto paramagnetic latex beads. Patient samples are loaded into a sample rack and then in the sample carousel of the instrument. A patient's serum is diluted by the instrument with sample dilution buffer in a disposable cuvette. A small amount of the diluted sample is combined with assay buffer and antigen coated beads in a second cuvette, and mixed. This reaction cuvette is incubated for 9 ½ minutes at 37°C. The cuvette is then exposed to a small magnet that holds the beads in place, the liquid is aspirated, and the beads are re-suspended as system rinse is added to the cuvette and the magnet is removed. This wash cycle is repeated one more time. During the third wash, no system rinse is added after the aspiration step. After the third wash, isoluminol conjugated monoclonal anti-human IgG (known as tracer IgG) is added to the beads in the cuvette, and mixed. Again, the cuvette is incubated for 9 ½ minutes at 37°C. Three wash steps, as described above, are performed on the beads. In the fourth wash step, no liquid is added to the beads after the aspiration. The cuvette is then placed in a light-tight luminometer and the beads are exposed to a base and an oxidizing agent. These two reagents, or "Triggers", cause the isoluminol to produce a flash of visible light. The light produced from this reaction is measured as RLU by the BIO-FLASH® optical system. The RLU are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which is in turn proportional to the amount of autoantibodies bound to the antigen on the beads.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*

      For each of the QUANTA Flash® assay, patient samples were evaluated, including samples close to the cutoff of 20 CU. The precision for within-run, between-day and between-run were evaluated.

      QUANTA Flash® PR3:

      Precision of the QUANTA Flash® PR3 assay was evaluated by running nine patient samples across the assay range. Samples were run in duplicates, twice a day, for 25 days over a 39 day period on one reagent lot (total of 100 replicates per sample).

Controls were run as quality control during each run. The study results are summarized in the table below:

N	Mean Value CU	Within Run %CV	Between Run %CV	Between Day %CV	Total Imprecision %CV
100	7.0	4.7	2.3	2.9	5.9
100	15.0	3.5	2.6	3.6	5.7
100	23.5	4.2	2.3	3.3	5.9
100	34.2	4.1	2.2	2.6	5.4
100	72.0	3.9	2.9	2.8	5.6
100	307.9	3.1	3.4	3.2	5.6
100	717.5	3.3	3.2	2.1	5.1
100	1,343.1	6.2	2.5	3.2	7.4
100	2,496.3	4.9	4.2	3.0	7.2

QUANTA Flash® MPO:

Precision of the QUANTA Flash® GBM assay was evaluated by running eight patient samples across the assay range. The samples were run in duplicates, twice a day, for 21 days over a 44 day period on one reagent lot (total of 84 replicates per sample). 3 additional samples were tested in duplicates for 20 days and 2 runs per day (total of 80 replicates per sample) Controls were run as quality control during each run. The study results are summarized in table below:

N	Mean Value CU	Within Run %CV	Between Run %CV	Between Day %CV	Total Imprecision %CV
84	12.7	10.4	0.0	7.4	12.8
80	17.6	3.8	0.9	3.1	5.5
80	19.0	3.8	0.0	4.0	5.5
80	20.2	3.9	0.0	3.2	5.1
84	32.4	4.3	0.0	3.8	5.8
84	36.0	3.9	0.0	5.8	7.0
84	39.0	6.0	0.0	8.5	10.4
84	41.5	5.3	0.0	4.4	6.9
84	124.1	5.0	0.0	3.9	6.4
84	641.0	4.5	0.0	4.4	6.3

QUANTA Flash® GBM:

Precision of the QUANTA Flash® GBM assay was evaluated by running eight patient samples across the assay range. The samples were run in duplicates, twice a day, for 21 days over a 36 day period on one reagent lot (total of 84 replicates per sample). Four additional samples were tested in duplicates for 20 days and 2 runs per day (total of 80 replicates per sample). Controls were run as quality control during each run. The study results are summarized in the table below:

N	Mean Value CU	Within Run %CV	Between Run %CV	Between Day %CV	Total Imprecision %CV
84	15.5	3.1	1.7	3.1	4.7
80	22.0	5.3	2.5	4.2	7.3
80	22.7	3.3	2.8	4.8	6.5
84	29.1	4.1	0.0	2.9	4.9
84	31.1	3.6	1.6	1.9	4.4
80	32.5	4.8	1.6	5.4	7.4
84	34.0	2.6	2.0	3.1	4.5
84	49.8	3.3	1.3	2.6	4.4
84	96.4	6.7	2.2	0.0	6.9
84	401.5	2.8	1.6	2.7	4.2
84	657.7	3.1	2.3	2.6	4.7
80	1,189.1	9.5	2.3	7.6	12.4

Lot-to-Lot Reproducibility was evaluated by testing samples with concentrations across the assay range in each assay. Samples with concentrations around the cut-off have CV<10%.

b. Linearity/assay reportable range:

The linear range was determined by diluting a high positive sample with a negative sample by the dilution scheme recommended in CLSI EP6-A. To cover the entire range several dilution series (starting at different points on the curve) were generated and tested. The expected value was plotted against the observed value, and linear regression analysis was performed to get slope, intercept and R^2 values. The results are summarized in the tables below:

QUANTA Flash® PR3:

Dilution range (CU)	Slope	Intercept	R^2
1.6 – 16.2	1.00 (0.97 to 1.03)	-0.03 (-0.34 to 0.28)	1.00
2.8 – 28.4	0.96 (0.92 to 1.01)	0.13 (-0.63 to 0.89)	0.99
6.5 – 65.3	1.01 (1.01 to 1.05)	0.35 (-1.14 to 1.84)	0.99
8.8 – 87.7	1.02 (0.98 to 1.05)	-1.33 (-3.13 to 0.48)	0.99
69.9 – 699.8	0.98 (0.98 to 0.94)	-4.03 (-18.27 to 10.22)	0.99
391.1 – 3,520.1	0.96 (0.94 to 0.99	-67.70 (-118 to -17.0)	1.00

The claimed linear range for PR3 IgG is 2.3 – 3,285.3 CU.

QUANTA Flash® MPO:

Dilution range (CU)	Slope	Intercept	R^2
1.3 – 12.5	0.99 (0.96 to 1.02)	0.0 (-0.26 to 0.2)	1.00
3.2 – 32.1	1.00 (0.97 to 1.02	0.6 (0.02 to 1.1)	1.00
3.4 – 34.1	0.99 (0.96 to 1.01)	-0.3 (-0.79 to 0.26)	1.00
5.7 – 56.7	1.02 (0.98 to 1.05)	-0.4 (-1.57 to 0.86)	0.99
6.3 – 63.0	1.00 (0.97 to 1.03)	-0.6 (-1.83 to 0.69)	0.99
6.5 – 64.7	1.04 (1.00 to 1.08)	1.1 (-0.49 to 2.61)	0.99
8.8 – 87.7	1.01 (0.98 to 1.04)	0.7 (-0.92 to 2.3)	1.00
32.3 – 322.5	1.02 (0.99 to 1.05)	2.6 (-3.45 to 8.7)	1.00
73.5 – 735.4	0.94 (0.94 to 0.98)	5.12 (-12.78 to 23.02)	0.99
93.9 – 939.0	0.97 (0.93 to 1.00)	32.0 (11.76 to 52.2)	0.99

The claimed linear range for MPO IgG is 3.2 – 739.8 CU.

QUANTA Flash® GBM:

Dilution range (CU)	Slope	Intercept	R^2
1.7 – 17.6	0.95 (0.91 to 1.00)	0.2 (0.29 to 0.69)	0.99
3.5 – 35.1	1.01 (0.97 to 1.05)	-0.3 (-1.14 to 0.59)	0.99
4.7 – 46.9	0.99 (0.95 to 1.04)	-0.2 (-1.35 to 0.94)	0.99
10.6 – 97.1	0.98 (0.95 to 1.01)	-1.3 (-2.89 to 0.39)	1.00
60.9 – 610.5	0.99 (0.96 to 1.01)	-0.7 (-9.89 to 8.56)	1.00
1.9 – 962.5	1.10 (1.06 to 1.13)	3.9 (-7.73 to 15.47)	1.00
155.9 – 1,558.6	0.93 (0.87 to 1.00)	22.6 (-37.80 to 83.08)	0.98

The claimed linear range for GBM IgG is 2.9 – 1,437.8 CU.

Reportable Range:

The reportable range of each assay is defined by the lowest and highest points on the Master Curve. The lowest point and the highest point on the curve of QUANTA Flash® PR3, MPO and GBM are shown in the table below:

Assay	Lowest point (CU)	Highest point (CU)
QUANTA Flash® PR3	2.3	3,285.3
QUANTA Flash® MPO	3.2	739.8
QUANTA Flash® GBM	2.9	1,437.8

Over the range Detection:

If the auto-rerun feature is not enabled by the customer, any patient with a calculated CU above the reportable range will be reported as greater than the highest point of the reportable range (e.g., >3,285.3 CU" for PR3). If the auto-rerun feature is enabled, the instrument will automatically perform a 1:20 dilution and rerun the sample. To assess the auto-rerun capability of the instrument, two samples greater than the highest point of the reportable range were run with the auto-rerun feature enabled. These samples were also diluted manually and retested. The manual dilution and the instrument re-run result variations were between 0% and 7% for the samples tested.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability:

There are no reference standards for IgG anti-PR3, anti-MPO or anti-GBM. Calibrators and controls are assigned values based on a 20 unit cutoff between positive and negative during assay development. Calibrators and control materials are specified in the labeling but are not supplied with the assay. The table below summarizes the control and calibrators target values:

	PR3	MPO	GBM
Calibrators			
Calibrator 1	10	10	10
Calibrator 2	580	190	400
Controls			
Control 1	10	10	10
Control 2	50	50	50

Calibrators:

The QUANTA Flash® PR3, MPO and GBM Calibrators are designed to adjust the predefined Master Curve into an instrument specific working curve. Two calibrators for each assay, one below the cut-off and one above the cut-off are manufactured and the target values are determined using the master curve generated using the six in-house standard points. Each calibrator has a specific CU. The BIO-Flash® instrument uses these unit values, together with the resulting RLU produced by each calibrator, to establish point of reference for the working curve. This working curve is used to calculate CU from RLU for each patient. Calibrators consist of diluted

human serum containing IgG to PR3, MPO or GBM. Stability is based on accelerated stability studies. The claimed calibrator's shelf life is 1 year at 2-8°C. The calibrators may be stored open for a maximum of 8 hours onboard the instrument. Real time stability is ongoing.

Controls:

Controls are manufactured by diluting human serum containing high-titer IgG anti-PR3, MPO or GBM antibodies into buffer. A target CU value is achieved through trial dilutions. Once a dilution is selected, the controls are tested, and adjusted. The product undergoes extensive final validation testing to assign a final value. Stability is based on accelerated stability studies. The claimed controls shelf life is 1 year at 2-8°C. Controls may be used up to 15 times, 10 minute per use onboard the instrument. Real time stability study is ongoing.

Kit Stability:

The claimed stability is based on accelerated studies and is summarized in the table below. Real time stability is ongoing.

	PR3	MPO	GBM
Kit – shelf life (2-8°C)	1 year	1 year	1 year
Kit – on board stability	70 days	86 days	42 days

d. Detection limit:

Limit of Blank (LoB):

Immunoglobulin stripped serum was used as the blank material. 30 samples were run at least twice, in replicates to obtain at least 60 measurements. The LoB RLU values are lower than the bottom limit of the four parameter logistic curve that the instrument used to calculate CU, and therefore cannot be converted into CU. The bottom limit of the curve is approximately 2000 RLU for all assays and is equivalent to 2.3 CU, 3.2 CU and 2.9 CU for PR3, MPO and GBM, respectively. The following equation was used to calculate the LoB: $LoB = \mu_B + 1.645 * \sigma_B$
The following LoB was determined:
LoB < 2.3 for PR3, < 3.2 CU for MPO and < 2.9 CU for GBM.

Limit of Detection (LoD):

Four sera for the PR3 and GBM CIAs and five sera for the MPO CIA were serially diluted until the RLU values no longer show a decreasing trend. Serum samples were diluted to a concentration that are between the LoB and approximately 4 x LoB. The samples were run in replicates of 4 for 4 days; 2 days on 1 instrument and 2 more days on another instrument to obtain 16 measurements per sample for a total of at least 60 measurements. The LoD for the three assays is below the lower limit of the reportable range.

e. Analytical specificity:

Interfering substances:

PR3, MPO and GBM sera with different analyte concentrations (negative, positive and around the cutoff) were mixed with known quantities of bilirubin (10, 5 or 2.5 mg/dL), hemoglobin (200, 100 or 50 mg/dL), cholesterol (224.3, 112.2 or 56.0 mg/dL), triglycerides (1000, 500 or 250 mg/dL) or rheumatoid factor (RF) (about 500, 300 or 100 IU/mL). No interference was observed up to the concentrations listed in the table below:

Potential Interfering Compound	Concentration
Bilirubin	10 mg/dL
Hemoglobin	200 mg/dL
Tryglycerides	1000 mg/dL
Cholesterol	224.3 mg/dL
Rheumatoid factor	500 IU/mL

Hook effect:

A separate experiment showed that the assay does not appear to demonstrate a hook effect at high concentrations: up to 8,919.7 CU for the PR3 assay, up to 5,744.5 CU for MPO and up to 6,701.7 CU for GBM.

f. Assay cut-off:

The cutoff is the same as the predicate and was set at:
Negative <20 CU
Positive ≥20 CU

2. Comparison studies:

a. Method comparison with predicate device:

QUANTA Flash® PR3:

181 samples were tested by QANTA Flash® PR3 and by the QUANTA Lite® PR-3 ELISA. Additional 18 contrived samples (±25% of the cut-off) were evaluated. Discrepant samples: 6 were from patients diagnosed with granulomatosis with polyangiitis (GPA) and 7 samples were from contrived samples around the cutoff. The results are summarized in the table below:

		QUANTA Lite® PR-3 (CU)		
		Positive	Negative	Total
QUANTA Flash® PR3 CIA	Positive	55	12	67
	Negative	1	131	132
	Total	56	143	199

Positive % agreement: 98.2% (55/56) (95% CI: 90.4 – 100.0%)
Negative % agreement: 91.6% (131/143) (95% CI: 85.8 – 95.6%)

Total % agreement: 93.5% [(55+131)/199] (95% CI: 89.1 – 96.5%)

QUANTA Flash® MPO:

167 samples were tested by QANTA Flash® MPO and by QUANTA Lite® MPO ELISA (predicate device). Additional 11 contrived samples (±25% of the cut-off) were evaluated. Discrepant samples: 11 were from patients with microscopic polyangiitis (MPA), 1 from a patient with Churg-Strauss syndrome (CSS), 3 from GPA patients, and 5 from contrived samples. The results are summarized in the table below:

		QUANTA Lite® MPO (CU)		
		Positive	Negative	Total
QUANTA Flash® MPO CIA	Positive	52	20	72
	Negative	0	106	106
	Total	52	126	178

Positive % agreement: 100.0% (52/52) (95% CI: 93.2 – 100.2%)
Negative % agreement: 84.1% (106/126) (95% CI: 76.6 – 90.0%)
Total % agreement: 88.8% [(52+106)/178] (95% CI: 83.2 – 93.0%)

QANTA Flash® GBM:

120 samples were tested by QANTA Flash® GBM and also by the QUANTA Lite® GBM ELISA (predicate device). Additional 18 contrived samples (±25% of the cut-off) were evaluated. Discrepant samples: 2 were from patients with Goodpasture's syndrome (GPS) and 8 from contrived samples. The results are summarized in the table below:

		QUANTA Lite® GBM (CU)		
		Positive	Negative	Total
QUANTA Flash® GBM CIA	Positive	91	2	93
	Negative	9	36	45
	Total	100	38	138

Positive % agreement: 91.0% (91/99) (95% CI: 83.6 – 95.8%)
Negative % agreement: 94.7% (36/38) (95% CI: 92.3 – 99.4%)
Total % agreement: 92.0% [(91+36)/138] (95% CI: 86.2 – 96.0%)

b. Matrix comparison:
Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

The clinical samples that were used for the method comparison were used to determine sensitivity and specificity of each assay. Diagnosis of ANCA-associated vasculitis (AAV) was made according to the American College of Rheumatology (ACR) and European study group (EUVAS) criteria, and the Chapel Hill Consensus Conference definitions (CHCC). Samples from normal blood donors were excluded

from the calculations. The sensitivity and specificity results are summarized below:
QUANTA Flash® PR3 CIA:

	Diagnostic Group – GPA		
	+	-	Total
Positive test ≥20 CU	69	15	84
Negative test <20 CU	32	570	602
Total	101	585	686

Sensitivity (95% CI): 68.3% (58.3 – 77.2%)
Specificity (95% CI): 97.4% (95.8 – 98.6%)
Total agreement (95% CI): 93.1% (91.0 – 94.9%)

QUANTA Flash® MPO CIA:

	Diagnostic Group – MPA		
	+	-	Total
Positive test ≥20 CU	71	22	93
Negative test <20 CU	18	550	568
Total	89	572	661

Sensitivity (95% CI): 79.8% (69.9 – 87.6%)
Specificity (95% CI): 96.2% (94.2 – 97.6%)
Total agreement (95% CI): 93.9% (91.9 – 95.6%)

QUANTA Flash® GBM CIA:

	Diagnostic Group – GPS		
	+	-	Total
Positive test ≥20 CU	86	1	87
Negative test <20 CU	4	396	400
Total	90	397	487

Sensitivity (95% CI): 95.6% (89.0 – 98.8%)
Specificity (95% CI): 99.7% (98.6 – 100.0%)
Total agreement (95% CI): 99.0% (97.6 – 99.7%)

The tables below show the results for each clinical subgroup and each analyte:

Prevalence of PR3-ANCA determined by QUANTA Flash® PR3:

Disease	n=	No (%) pos
Granulomatosis with polyangiitis (GPA)	101	69 (68.3%)
Microscopic Polyangiitis (MPA)*	73	2 (2.7%)
Churg-Strauss Syndrome (CSS)*	10	0 (10%)
Goodpasture`s syndrome (GPS)*	68	0 (0.0%)
Normals	405	3 (0.74%)
Connective tissue disease (CTD)	100	0 (0.0%)
Systemic lupus erythematosus (SLE)	43	0 (0.0%)
Systemic sclerosis (SSc)	50	0 (0.0%)

Sjoegren Syndrome (SjS)	7	0 (0.0%)
Infectious diseases	99	3 (3.0%)
Hepatitis C virus infection (HCV)	47	0 (0.0%)
Hepatitis B virus infection (HBV)	32	1 (3.1%)
Human immunodeficiency virus infection (HIV)*	10	2 (20.0%)
Syphilis	10	0 (0.0%)
Other diseases	235	10 (4.3%)
Rheumatoid arthritis (RA)	64	0 (0.0%)
Multiple sclerosis	20	0 (0.0%)
Inflammatory bowels disease (IBD)*	70	8 (11.4%)
Other disease group	81	2 (2.5%)
Total (not including 405 normal samples)	686	

*CSS, MPA, HIV and IBD patients are known to contain anti-PR3 antibodies.

Prevalence of MPO-ANCA determined by QUANTA Flash® MPO:

Disease	n=	No (%) pos
Microscopic Polyangiitis (MPA)	89	71 (79.8%
Churg-Strauss Syndrome (CSS)*	10	1 (10%)
Granulomatosis with polyangiitis (GPA)	89	8 (9.0%)
Goodpasture`s syndrome (GPS)*	47	10 (19.2%)
Normals	405	0 (0.0%)
Connective tissue disease (CTD)	157	0 (0.6%)
Systemic lupus erythematosus (SLE)	101	1 (1.0%)
Systemic sclerosis (SSc)	49	0 (0.0%)
Sjoegren Syndrome (SjS)	7	0 (0.0%)
Infectious diseases	65	0 (0.0%)
Hepatitis C virus infection (HCV)	19	0 (0.0%)
Hepatitis B virus infection (HBV)	29	0 (0.0%)
Human immunodeficiency virus infection (HIV)	7	0 (0.0%)
Syphilis	10	0 (0.0%)
Other diseases	204	2 (1.0%)
Rheumatoid arthritis (RA)	73	0 (0.0%)
Multiple sclerosis	18	0 (0.0%)
Inflammatory bowels disease (IBD)	47	2 (4.3%)
Other disease group	66	0 (0.0%)
Total (not including 405 normal samples)	661	

*CSS and GPS patients are known to contain anti-MPO antibodies.

Prevalence of anti-GBM antibodies determined by QUANTA Flash® GBM:

Disease	n=	No (%) pos
Goodpasture’s syndrome (GPS)	90	86 (95.6%)
Healthy donors	400	2 (0.5%)
Rheumatoid arthritis	50	0 (0.0%)
Systemic lupus erythematosus (SLE)	67	0 (0.0%)
Undifferentiated inflammatory bowel disease	50	0 (0.0%)

Systemic sclerosis (SSc)	50	0 (0.0%)
Multiple sclerosis (MS)	20	0 (0.0%)
Undifferentiated connective tissue disease (CTD)	10	0 (0.0%)
Ulcerative colitis	10	0 (0.0%)
Hepatitis B virus	32	0 (0.0%)
Hepatitis C virus	58	0 (0.0%)
Human immunodeficiency virus (HIV)	10	1 (10.0%)
Syphilis	10	0 (0.0%)
Other disease group	30	0 (0.0%)
Total (not including 400 normal samples)	487	

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off**:**
Same as assay cut-off.

5. Expected values/Reference range:
405 samples from random blood donors were tested. The value was found to be:
PR3 - The average was 3.3 CU and the 95% percentile was calculated as 4.9 CU;
MPO - The average was <3.2 CU and the 95% percentile was calculated as <3.2 CU;
GBM - The average was 3.3 CU and the 95% percentile was calculated as 5.1 CU.

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.